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Mouse Monoclonal Antibodies Reactive with J5 Lipopolysaccharide Exhibit Extensive Serological Cross-Reactivity with a Variety of Gram-Negative Bacteria

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We describe two mouse monoclonal antibodies reactive with lipopolysaccharide derived from the J5 mutant of *Escherichia coli* O111:B4. These antibodies react with purified lipopolysaccharide derived from rough mutants of *E. coli* and *Salmonella typhimurium* and also with lipopolysaccharide associated with both smooth- and rough-phenotype, gram-negative bacteria. Both antibodies appear to bind determinants present in the lipopolysaccharide core region, and this reactivity is inhibited in the presence of polymyxin B. Although their patterns of reactivity differ, both antibodies exhibit extensive serological cross-reactivity with a variety of gram-negative bacteria. Reagents of this type should prove useful in animal models to delineate the requisite affinity, epitope specificity, immunoglobulin class, etc., needed for the prevention and treatment of gram-negative bacteremia.

A number of studies in animal models (1–3, 11, 12, 17, 18, 24) and in humans (25) have indicated that antibodies specific for the core region of lipopolysaccharide (LPS) are cross-reactive with LPS derived from a variety of gram-negative organisms. In these studies, rough mutants of *Escherichia coli* and *Salmonella minnesota*, or the LPS derived from them, were employed for the production of anti-core antibodies. These mutants possess enzyme defects that render them unable to incorporate the immunodominant oligosaccharide side chains onto the core region of the LPS molecule. Antibodies reactive with LPS core determinants have been shown to protect against the lethal effects of LPS or gram-negative infection from *E. coli* (2, 18, 24, 25), *Salmonella typhosa* (12), *Klebsiella pneumoniae* (24), *Pseudomonas aeruginosa* (1), *Neisseria meningitidis* (3), and *Haemophilus influenzae* (17). Anti-core antibodies are thought to offer protection against gram-negative infection and endotoxemia by virtue of the known structural similarities among the core regions of serologically unique LPS molecules (16).

In this report we describe the production and characterization of mouse monoclonal antibodies reactive with core determinants present on LPS molecules derived from the J5 mutant of *E. coli* O111:B4. We describe the fine specificity of these antibodies and demonstrate their extensive serological cross-reactivity on LPS associated with gram-negative clinical isolates. These antibodies should be useful in future studies aimed at determining the therapeutic and diagnostic utility of monoclonal anti-core antibodies.

MATERIALS AND METHODS

Reagents. LPS was purchased from List Biological Laboratories, (Campbell, Calif.) or Ribi ImmunoChem Research, Inc. (Hamilton, Mont.) or were obtained from Thomas Tachovsky (Cambridge Research Laboratory) and Richard Ulevitch (Scripps Clinic and Research Foundation). LPS derived from rough and smooth strains of bacteria were prepared by established methods (6, 23). Polymyxin B sulfate was purchased from Sigma Chemical Co. (St. Louis, Mo.). Bovine serum albumin (BSA) and keyhole limpet hemocyanin (KLH) were haptenated with *p*-arsanilic acid (Ar) by published methods (14). Carrier-free $Na^{125}I$ was purchased from Amersham Corp. (Arlington Heights, Ill.).

Production of hybridomas. BALB/c mice (Jackson Laboratory, Bar Harbor, Maine) were immunized with the galactose epimerase-deficient J5 mutant of *E. coli* O111:B4 (4). Lymphocytes were fused with the mouse myeloma cell line P3-X63-Ag8.653 (13) at a 5:1 lymphocyte/tumor ratio with polyethylene glycol (PEG 1000; J. T. Baker Chemical Co., Phillipsburg, N.J.) as described by Gefter et al. (8). Cells were plated in 96-well microtiter trays, and hybrids were selected in hypoxanthine-aminopterin-thymidine medium by the method of Littlefield (15) and cloned by limiting dilution. Hybridoma 24-8-8G was obtained from C57BL/6J mice after immunization with purified O55:B5 LPS and fusion of spleen cells with the SP2/0-Ag14 hybridoma cell line (22). Hybridoma 2-18-11 was obtained from A/J strain mice after immunization with KLH-Ar and fusion of spleen cells with SP2/0-Ag14. Both hybrids were selected and cloned as described above.

Hybridomas were screened for antibody activity by an enzyme-linked immunosorbent assay (ELISA) in flexible 96-well polyvinyl chloride (PVC) microtiter plates coated with the relevant antigens. Anti-J5 monoclonal antibodies were purified on protein A-Sepharose (Pharmacia Fine Chemicals, Piscataway, N.J.) affinity columns as described by Ey et al. (5). Anti-Ar monoclonal antibody (MAb) 2-18-11 was affinity purified on BSA-Ar-Sepharose columns as described by Lamoyi et al. (14). Hybridoma immunoglobulin subclass determinations were performed by ELISA with reagents purchased from Zymed Laboratories, Inc. (South San Francisco, Calif.). Anti-J5 MAb 48-1-10C and MAb 49-2-11B and anti-Ar MAb 2-18-11 possess the immunoglobulin G2b (IgG2b) isotype. Anti-O55:B5 LPS MAb 24-8-8G possesses the IgM isotype.

Antibody assays. Reactivity and specificity of anti-J5 MAb 48-1-10C and MAb 49-2-11B were determined by ELISA with purified J5 LPS or heat-killed bacteria as solid-phase antigen in 96-well PVC microtiter trays. Bound mouse

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TABLE 1. Reactivity of anti-J5 monoclonal antibodies with J5 LPS and *E. coli* cells

Solid-phase antigen[a]	Optical density values[b]		
	Medium	48-1-10C	49-2-11B
E. coli J5 LPS	0.035	1.391	1.504
E. coli J5 cells	0.007	0.620	1.462
E. coli O111:B4 cells	0.033	0.948	1.783

[a] PVC microtiter trays (96 wells) were coated with purified LPS or heat-killed bacteria as described in the text.

[b] Culture medium and hybridoma culture supernatants 48-1-10C and 49-2-11B were added to PVC wells and incubated for 3 h at room temperature. Antibody binding was assessed with horseradish peroxidase-conjugated goat-anti-mouse immunoglobulin and orthophenylene diamine as described in the text.

monoclonal antibody was detected with horseradish peroxidase-conjugated goat anti-mouse immunoglobulin (New England Nuclear Corp., Boston, Mass.). Orthophenylene diamine was used as the substrate, and color development was terminated with 4.5 M H_2SO_4. Plates were read in a Dynatech MR 600 microplate reader at 490 nm.

ELISA inhibition assays employed purified LPS or BSA-Ar as the solid-phase antigen. Inhibitors were added before limiting amounts of monoclonal antibody. The percent inhibition of binding was determined as: (OD_{490} without inhibitor − OD_{490} with inhibitor/OD_{490} without inhibitor) × 100, where OD_{490} is the optical density at 490 nm.

Competition assays employed J5 LPS as the solid-phase antigen and 20 ng of ^{125}I-labeled, protein A-purified MAb 48-1-10C and MAb 49-2-11B. Proteins were radioiodinated as described by Hunter (10). The percent inhibition of binding was determined as: (cpm bound in absence of inhibitor − cpm bound with inhibitor/cpm bound in absence of inhibitor) × 100, where cpm is counts per minute.

Bacteria. The J5 mutant of *E. coli* strain O111:B4 was obtained from Thomas Tachovsky (Cambridge Research Laboratory). *E. coli* strain O111:B4 was obtained from Robert Arbeit (Veterans Administration Medical Center, Boston, Mass.). Gram-negative clinical isolates were obtained from Debra Buxton (Cambridge Research Laboratory). Bacteria were grown in brain heart infusion broth (Scott Laboratories, Fiskeville, R.I.) overnight, washed in phosphate-buffered saline, and heat killed for 2 h at 100°C. After dilution to appropriate concentrations in 0.25% glutaraldehyde–phosphate-buffered saline, bacterial suspensions were used to coat 96-well microtiter trays.

RESULTS

Production and screening of anti-J5 MAb. BALB/c mice were immunized with the J5 mutant of *E. coli* O111:B4. J5 cells lack the enzyme uridine 5′-diphosphate-galactose 4-epimerase and are unable to synthesize complete LPS molecules possessing oligosaccharide side chains (4). LPS obtained from the J5 mutant is comprised solely of lipid A, 3-deoxy-D-manno-octulosonic acid (KDO), ethanolamine, phosphate, heptose, and glucose. After fusion of immune lymphocytes with the mouse myeloma cell line P3-X63-Ag.8.653, two hybridomas were identified which produced antibodies reactive with purified J5 LPS.

Culture supernatants derived from cloned hybridoma cell lines 48-1-10C and 49-2-11B reacted strongly with purified J5 LPS, rough strain J5 bacteria, and smooth parental strain O111:B4 bacteria (Table 1). Purified J5 LPS obtained from three different sources gave comparable results in the ELISA (data not shown). Radioimmunoassays employing ^{125}I-labeled monoclonal antibodies and either J5 LPS coated on PVC wells at a concentration of 25 μg/ml or smooth, heat-killed O111:B4 cells in suspension showed that about 50% of the added 25 ng of radiolabeled antibody bound to solid-phase J5 LPS and approximately 3,000 antibodies bound per heat-killed bacterium.

Table 2 shows the results of inhibition assays with anti-J5 monoclonal antibodies in conjunction with a variety of purified LPS preparations. J5 LPS inhibited the binding of both monoclonal antibodies to the homologous solid phase antigen. MAb 49-2-11B required approximately 100-fold less J5 LPS than that required by MAb 48-1-10C for a comparable level of inhibition (77% inhibition with 0.02 μg versus 65% inhibition with 2.5 μg, respectively). LPS derived from *E. coli* O111:B4 was non-inhibitory in the ELISA.

LPS derived from an Re mutant of *Salmonella typhimurium*, G30/C21, inhibited the reactivity of both anti-J5 monoclonal antibodies, and the level of inhibition was equivalent to that obtained with J5 LPS. MAb 49-2-11B required approximately 100-fold less Re LPS than did MAb 48-1-10C to achieve comparable levels of inhibition. LPS derived from the smooth parental strain *S. typhimurium* was non-inhibitory in a fashion similar to that observed with O111:B4 LPS.

The results presented in Tables 1 and 2 indicate that, although both anti-J5 monoclonal antibodies react with J5 *E. coli* LPS and with LPS derived from an Re mutant of *S. typhimurium*, they are not reactive with LPS derived from either the parental, smooth homologous strain (O111:B4 *E. coli*) or heterologous strain (*S. typhimurium* wild type).

Competition assays were performed to ascertain whether the two anti-J5 monoclonal antibodies reacted with the same epitope. Wells of PVC trays were coated with J5 LPS, and unlabeled antibody preparations were utilized to inhibit the binding of 20 ng of radiolabeled MAb 48-1-10C or MAb 49-2-11B with the solid-phase antigen (Table 3). Although both anti-J5 monoclonal antibodies inhibited each other's reactivity, MAb 49-2-11B was a more effective inhibitor, on a weight basis, than MAb 48-1-10C in both the homologous and heterologous competition assays. Unlabeled MAb 49-2-11B was able to completely displace labeled MAb 48-1-10C (97% inhibition at 62.5 ng), whereas unlabeled MAb 48-1-10C was unable to effect 50% inhibition of binding of radiolabeled MAb 49-2-11B (39% inhibition at 1,000 ng). These results are most likely due to affinity differences

TABLE 2. Comparison of reactivity of monoclonal antibodies with LPS derived from smooth- and rough-strain bacteria

Inhibitor[a]	Amt (μg)	% Inhibition of binding	
		49-2-11B	48-1-10C
E. coli J5 LPS	0.004	49	5
	0.02	77	4
	0.10	88	10
	0.50	96	37
	2.50	98	65
E. coli O111:B4 LPS	2.50	2	0
S. typhimurium Re LPS	0.004	23	ND[b]
	0.02	78	ND
	0.10	98	ND
	0.50	100	ND
	2.50	100	76
S. typhimurium wild type LPS	2.50	0	0

[a] Inhibition assays utilized PVC wells coated with purified J5 LPS. Various amounts of purified LPS were used to inhibit the binding of anti-J5 monoclonal antibodies as described in the text.

[b] ND, Not done.

TABLE 3. Competition assay between MAb 48-1-10C and MAb 49-2-11B[a]

Unlabeled inhibitor[b]	Amt (ng)	% Inhibition of binding	
		48-1-10C[c]	49-2-11B[c]
48-1-10C	16	30	6
	63	75	26
	250	82	15
	1,000	92	39
49-2-11B	16	88	56
	63	97	72
	250	100	92
	1,000	100	98
2-18-11	1,000	3	14

[a] PVC trays were coated with limiting amounts of J5 LPS. Unlabeled monoclonal antibodies were added immediately before 20 ng of radiolabled MAb 48-1-10C or MAb 49-2-11B.
[b] Inhibitors 48-1-10C and 49-2-11B were protein A purified; anti-Ar MAb 2-18-11 was affinity purified on BSA-Ar.
[c] Radiolabeled ligand.

between the two monoclonal antibodies. The LPS inhibition data presented in Table 2 also support this interpretation.

Polymyxin B inhibits reactivity between J5 LPS and anti-J5 monoclonal antibodies. Experiments were performed to assess the ability of the cationic antibiotic polymyxin B to inhibit the reactivity of J5 LPS with MAb 48-1-10C and MAb 49-2-11B. Studies by Morrison and Jacobs (19) and Schindler and Osborn (21) have shown that polymyxin B binds to the KDO as well as with the lipid A region of the LPS molecule. Polymyxin B specifically inhibited the binding of MAb 48-1-10C and MAb 49-2-11B to J5 LPS (Table 4). MAb 48-1-10C is somewhat more inhibitable by polymyxin B than MAb 49-2-11B as judged by the amounts of polymyxin B required for comparable levels of inhibition (37% inhibition at 0.125 μg versus 44% inhibition at 0.625 μg, respectively). These results are consistent with the data presented in Tables 2 and 3, which suggested the higher affinity of MAb 49-2-11B compared with that of MAb 48-1-10C.

Two additional monoclonal antibodies were utilized as controls for the polymyxin B inhibition studies. Antibodies derived from hybridoma 24-8-8G react with the O-somatic antigen of O55:B5 LPS, whereas antibodies derived from hybridoma 2-18-11 are of the same immunoglobulin subclass as the anti-J5 antibodies (IgG2b) and react with the Ar hapten. Polymyxin B did not inhibit the binding of either control monoclonal antibody (Table 4). The ability of LPS derived from smooth *E. coli* strain O55:B5 to partially inhibit

TABLE 4. Inhibition of binding of monoclonal anti-J5 antibodies by polymyxin B

Inhibitor[a]	Amt (μg)	% Inhibition of binding			
		48-1-10C	49-2-11B	24-8-8G	2-18-11
Polymyxin B	3.125	100	91	9	0
	0.625	80	44	10	ND[b]
	0.125	37	4	5	ND
J5 LPS	2.50	51	100	0	1
O55:B5 LPS	2.50	0	38	84	2
BSA-Ar	2.50	9	5	10	100

[a] PVC trays were coated with J5 LPS, O55:B5 LPS, or BSA-Ar. Polymyxin B was added to the wells before the addition of monoclonal antibodies. MAb 48-1-10C and MAb 49-2-11B are reactive with J5 LPS, MAb 24-8-8G is reactive with O55:B5 LPS somatic antigens, and MAb 2-18-11 is reactive with Ar.
[b] ND, Not done.

TABLE 5. Reactivity of monoclonal anti-J5 antibodies with *E. coli* clinical isolates

Bacteria	Optical density values[a]	
	48-1-10C	49-2-11B
J5	0.681	0.813
O111:B4	0.674	0.814
1	0.503	0.495
2	0.052	0.100
3	0.123	0.091
4	0.390	0.289
5	0.033	0.074
6	0.265	0.352
7	0.563	0.553
8	0.123	0.151
9	0.075	0.050
10	0.064	0.052
11	0.263	0.031
12	0.012	0.049
13	0.137	0.057
14	0.174	0.278

[a] PVC trays were coated with heat-killed bacteria as described in the text. The reactivity of monoclonal antibodies was determined by ELISA.

the reactivity of MAb 49-2-11B with the J5 LPS was observed consistently (Table 4, 38% inhibition with 2.5 μg of O55:B5 LPS).

Presence of a conserved epitope(s) on LPS associated with gram-negative clinical isolates. A variety of functional and serological studies with polyclonal anti-J5 LPS antibodies have suggested the existence of a conserved core epitope(s) present on LPS derived from a variety of serologically unique gram-negative bacteria (1–3, 11, 12, 17, 18, 24). To determine whether the two anti-J5 monoclonal antibodies had an equivalent degree of cross-reactivity, we tested MAb 48-1-10C and MAb 49-2-11B against a variety of gram-negative clinical isolates. The results are shown in Table 5 for *E. coli*. Although there was a certain degree of day-to-day variability in the whole organism ELISA, the data shown are representative of those observed on a number of occasions. In our hands, ELISA optical density values of ≥0.100 are considered to represent positive reactivity of antibody with heat-killed organisms. In most cases, the reactivity of both antibodies with the various clinical isolates of *E. coli* paralleled one another. One exception is *E. coli* clinical isolate no. 11, which appeared to be more reactive with MAb 48-1-10C than with MAb 49-2-11B. In general, the reactivity of both monoclonal antibodies was somewhat lower with the clinical isolates than with the J5 bacterial cells or the parental strain O111:B4 cells. Culture media and a hybridoma culture supernatant containing anti-Ar MAb 2-18-11 were completely unreactive in the assay (data not shown).

Both anti-J5 monoclonal antibodies were assayed against a variety of non-*E. coli*, gram-negative clinical isolates (Table 6). In most instances, MAb 48-1-10C reacted significantly better than MAb 49-2-11B. In the case of *K. pneumoniae*, *Pseudomonas* spp., *Proteus* spp., *Enterobacter* spp., *Salmonella* spp., and *Acinetobacter* spp., MAb 48-1-10C usually reacted (14 of 16 tested), whereas MAb 49-2-11B reacted marginally, if at all (optical density values less than 0.1). Both antibodies reacted equally well with *Serratia marcescens* and *Shigella* spp. Clinical isolates of *Staphylococcus aureus* were also tested by ELISA as a negative control and were found to be unreactive with either anti-J5 monoclonal antibody (data not shown).

TABLE 6. Reactivity of anti-J5 monoclonal antibodies with non-*E. coli* gram-negative clinical isolates

Bacteria	Optical density values[a]	
	48-1-10C	49-2-11B
K. pneumoniae		
1	0.178	0.002
2	0.228	0.011
3	0.208	0.009
4	0.232	0.017
5	0.257	0.041
Pseudomonas sp.		
1	0.030	0
2	0.071	0
3	0.257	0.045
4	0.195	0.038
Proteus sp.		
1	0.270	0.054
2	0.231	0.028
3	0.242	0.042
4	0.282	0.057
S. marcescens	0.146	0.120
Enterobacter spp.	0.198	0.021
Salmonella spp.	0.150	0.066
Shigella spp.	0.241	0.205
Acinetobacter spp.	0.213	0.061

[a] PVC trays were coated with heat-killed bacteria as described in the text. The reactivity of monoclonal antibodies was determined by ELISA.

DISCUSSION

The purpose of the present study was to produce monoclonal antibodies reactive with LPS core structures common among *Enterobacteriaceae*. A number of points can be made concerning the serological reactivity of the two anti-J5 monoclonal antibodies described in this report. These antibodies react with purified, rough LPS and with LPS associated with both rough and smooth heat-killed organisms (Table 1), but are unreactive with purified LPS derived from smooth strain organisms (Table 2). Even LPS derived from the J5 parental strain, *E. coli* O111:B4, is unreactive with both antibodies.

The inability of anti-J5 MAb to react with smooth-strain LPS is most likely the result of the core epitopes being blocked by oligosaccharide side chains present to various degrees on wild-type LPS. Since these antibodies are reactive with LPS when associated with heat-killed, smooth-strain organisms (Tables 1, 5, and 6), it is apparent that this method of cell preparation somehow helps to unmask LPS core determinants present on the surface of bacteria. It is unclear at present whether heat treatment of smooth organisms exposes core determinants present on those LPS molecules possessing a full complement of oligosaccharide side chains or whether it simply affords accessibility to those LPS molecules present at a stage in assembly when they have not yet become associated with the side chains (9, 20). Heat treatment of purified, smooth LPS was unsuccessful in exposing core determinants as determined by direct binding studies (heat-treated LPS as a solid phase antigen) and inhibition studies (heat-treated LPS as an inhibitor of binding of MAb with solid phase J5 LPS) (M. Nelles, unpublished observations).

J5 LPS is characterized as being of the Rc chemotype and therefore contains ethanolamine, KDO, phosphate, heptose, glucose, and lipid A (4). The data presented in Table 3 demonstrate that neither heptose nor glucose contributes to the reactivity with either MAb since LPS derived from an Re mutant of *S. typhimurium* (which is comprised solely of KDO, phosphate, ethanolamine, and lipid A) is equivalent quantitatively to J5 LPS in its reactivity with both antibodies (Table 2).

The data shown in Table 4 indicate that polymyxin B specifically inhibits the interaction between the anti-core monoclonal antibodies and J5 LPS. Control experiments indicated that the inhibition resulted from the polymyxin B binding to LPS rather than to the anti-LPS antibodies. In addition, polymyxin B exerted no effect on the reactivity of a monoclonal antibody specific for the O-somatic antigens of O55:B5 LPS.

Although polymyxin B is known to react with both the lipid A region of LPS (19) and with the KDO present in the inner core region (21), we favor the interpretation that both monoclonal antibodies are reactive with the core portion of the LPS molecule. First, a number of studies have indicated that one must immunize animals with acid-hydrolyzed lipid A coated onto bacteria to produce an antibody response against lipid A (2, 7, 11). Immunization with either purified rough LPS or rough organisms results in an antibody response directed solely toward the non-lipid A region of the molecule. The mice from which both hybridomas were derived had been immunized with J5 cells. Second, experiments employing lipid A as either a solid-phase antigen in direct binding studies, or as an inhibitor to prevent reactivity of anti-core antibodies with solid phase J5 LPS, were uniformly negative (M. Nelles, unpublished observations).

The data presented in Table 3 seem to indicate, upon first inspection, that both antibodies are reactive with the same core epitope by virtue of the ability of MAb 49-2-11B to inhibit radiolabeled MAb 48-1-10C from binding to solid-phase J5 LPS. These results can also be explained by two antibodies possessing different affinities and reacting with distinct core epitopes located in close proximity to one another. Although both antibodies show qualitatively similar patterns of reactivity against the *E. coli* clinical isolates tested (Table 5), their patterns of reactivity against non-*E. coli* clinical isolates are quite different (Table 6). Based on these results, we favor the idea that MAb 48-1-10C and 49-2-11B react with different core determinants.

Although the anti-J5 monoclonal antibodies described in this report do not react with purified LPS preparations containing oligosaccharide side chains (i.e., "smooth" LPS), they are reactive with LPS associated with laboratory strains (Table 1) and clinical isolates (Tables 5 and 6) of gram-negative bacteria. Both antibodies exhibit comparable patterns of serological reactivity against *E. coli* clinical isolates (Table 5). Studies utilizing a variety of non-*E. coli*, gram-negative clinical isolates (Table 6) demonstrated the preferential reactivity of one of the antibodies (MAb 48-1-10C). Despite the different patterns of reactivity, it is clear that both monoclonal antibodies react with epitopes that are conserved among members of the *Enterobacteriaceae*. Studies are in progress to more fully assess the extent of the cross-reactivity among gram-negative organisms and to determine the structural basis for the differences in reactivity between MAb 48-1-10C and MAb 49-2-11B. In addition, experiments are underway which are designed to determine the reasons for anti-J5 monoclonal antibodies reacting with smooth LPS associated with heat-killed organisms, but not with purified smooth LPS.

Our inability to demonstrate serological reactivity of anti-core antibodies with smooth LPS is not a new finding. Previous studies (10) as well as our own work (M. Nelles, unpublished observations) indicate quite clearly that polyclonal antisera and affinity-purified antibodies are unreactive with smooth LPS. In fact, the basis for the idea that anti-core antibodies are broadly cross-reactive derives from functional studies utilizing animals infected with a variety of gram-negative organisms or animals given purified smooth LPS. Although our studies do not resolve the conflict between serology and animal protection studies, they do indicate that monoclonal anti-core antibodies react with LPS in a fashion equivalent to that of polyclonal antibodies that have been used effectively in a therapeutic manner both in animal models (1–3, 11, 12, 17, 18, 24) and in humans (25). In conclusion, the results presented in this report lend support to the concept of conserved core determinants present in LPS molecules derived from a wide variety of gram-negative bacteria. This is, to our knowledge, the first published report in which monoclonal antibodies are used to demonstrate the extent of serological cross-reactivity among LPS present on *Enterobacteriaceae*. Although monoclonal anti-core antibodies of mouse origin might be expected to find limited usage in humans, these reagents should prove ideal in delineating the requisite affinity, epitope specificity, immunoglobulin class, etc., for the prevention and treatment of gram-negative bacteremia. Such information will be useful in preselecting human monoclonal antibodies for use in the therapeutics of gram-negative infection.

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ADDENDUM IN PROOF

A recent report by Mutharia et al. (L. M. Mutharia, G. Crockford, W. C. Bogard, Jr., and R. E. W. Hancock, Infect. Immun. 45:631–636) has also described mouse monoclonal antibodies reactive with epitopes shared by J5 lipopolysaccharide and lipopolysaccharide derived from a variety of gram-negative bacteria. Although the fine specificity of these monoclonal antibodies (anti-lipid A) differs from these described in our study (anti-core), the results of both studies lend support to the idea that one or more conserved epitopes are present on lipopolysaccharide molecules derived from serologically different gram-negative organisms.

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